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TO THE MEN AND WOMEN
OF OUR TIME AND COUNTRY WHO BY WISE AND GENEROUS GIVING
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IN ALL DEPARTMENTS OF KNOWLEDGE

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THE UNIVERSITY OF CHICAGO
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INVESTIGATIONS REPRESENTING THE DEPARTMENTS

ZOÖLOGY ANATOMY PHYSIOLOGY NEUROLOGY
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THE DECENNIAL PUBLICATIONS
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CONTENTS

I. On the Production and Suppression of Muscular Twitchings and Hypersensitiveness of the Skin by Electrolytes - - - - 1

By Jacques Loeb, Professor and Head of the Department of Physiology

II. On a Formula for Determining the Weight of the Central Nervous System of the Frog from the Weight and Length of its Entire Body - - - - - - - - - - 15

By Henry H. Donaldson, Professor and Head of the Department of Neurology

III. The Development of the Colors and Color Patterns of Coleoptera, with Observations upon the Development of Color in Other Orders of Insects (with Plates I–III) - - - 31

By William Lawrence Tower, Assistant in Embryology

IV. The Artificial Production of Spores in Monas by a Reduction of the Temperature - - - - - - - - - - 71

By Arthur W. Greeley, Assistant in Physiology

V. The Self-Purification of Streams - - - - - - - - 79

By Edwin Oakes Jordan, Associate Professor of Bacteriology

VI. The Lecithans: Their Function in the Life of the Cell - 91

By Waldemar Koch, Assistant in Pharmacology

VII. A Contribution to the Physical Analysis of the Phenomena of Absorption of Liquids by Animal Tissues - - - - 103

By Ralph Waldo Webster, Assistant in Physiological Chemistry

VIII. The Distribution of Blood-Vessels in the Labyrinth of the Ear of Sus Scrofa Domesticus (with Plates V–XII) - - - 135

By George E. Shambaugh, Instructor in Anatomy of the Ear, Nose, and Throat

127055

IX. THE ANIMAL ECOLOGY OF THE COLD SPRING SAND SPIT, WITH REMARKS ON THE THEORY OF ADAPTATION - - - - - - - - 155

By CHARLES BENEDICT DAVENPORT, Associate Professor of Zoölogy and Embryology

X. THE FINER STRUCTURE OF THE NEURONES IN THE NERVOUS SYSTEM OF THE WHITE RAT (with Plates XIII, XIV) - - - - - 177

By SHINKISHI HATAI, Research Assistant in Neurology

XI. THE PHYLOGENY OF ANGIOSPERMS - - - - - - - - - 191

By JOHN MERLE COULTER, Professor and Head of the Department of Botany

XII. STUDIES IN FAT NECROSIS - - - - - - - - - - 197

By H. GIDEON WELLS, Instructor in Pathology

XIII. OOGENESIS IN SAPROLEGNIA (with Plates XV, XVI) - - - 225

By BRADLEY MOORE DAVIS, Assistant Professor of Botany [HULL BOTANICAL LABORATORY]

XIV. THE EARLY DEVELOPMENT OF LEPIDOSTEUS OSSEUS (with Plates XVII, XVIII) - - - - - - - - - - - - 259

By ALBERT CHAUNCEY EYCLESHYMER, Assistant Professor of Anatomy

XV. THE STRUCTURE OF THE GLANDS OF BRUNNER (with Plates XIX–XXIV) - - - - - - - - - - - - - - 277

By ROBERT RUSSELL BENSLEY, Assistant Professor of Anatomy

XVI. MITOSIS IN PELLIA (with Plates XXV–XXVII) - - - - - 327

By CHARLES JOSEPH CHAMBERLAIN, Instructor in Morphology and Cytology

XVII. A DESCRIPTION OF THE BRAINS AND SPINAL CORD OF TWO BROTHERS DEAD OF HEREDITARY ATAXIA. (CASES XVIII AND XX OF THE SERIES IN THE FAMILY DESCRIBED BY DR. SANGER BROWN); (with plates XXVIII–XXXIX) - - - - - - - - - - 347

By LEWELLYS FRANKLIN BARKER, Professor and Head of the Department of Anatomy. With an Introduction by DR. SANGER BROWN

THE PRODUCTION OF MUSCULAR TWITCHINGS

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ON THE PRODUCTION AND SUPPRESSION OF MUSCULAR TWITCHINGS AND HYPERSENSITIVENESS OF THE SKIN BY ELECTROLYTES

JACQUES LOEB

IT has been shown in former publications that a slight variation in the proportion and character of the electrolytes in a tissue is capable of imparting to that tissue properties which it does not possess ordinarily, and it has been suggested that this fact might help us in recognizing the nature of a number of nervous and muscular diseases, and also possibly furnish a means of curing or mitigating them.[1] This paper contains some further contributions to the same subject. It deals with the determination of electrolytes which are liable to produce and inhibit hyperactivity of muscles and hypersensitiveness of the nerves of the skin; and tries to answer the question whether or not the stimulating and inhibiting effects of ions are a function of their valency and electrical charge.

I. THE PRODUCTION AND SUPPRESSION OF MUSCULAR TWITCHINGS BY ELECTROLYTES

1. Our muscles do not normally contract or twitch rhythmically, but they do so in certain diseases. The main electrolyte in our blood is sodium chloride. When we put a muscle into a pure sodium-chloride solution of the right osmotic pressure (*i. e.*, isotonic with the muscle), the muscle soon begins to twitch rhythmically, and these twitchings may last for several days, or about as long as the muscle lives. But when we add a very small, though definite, amount of a soluble calcium salt, the twitchings will not occur, though the muscle lives longer in such a solution than in a pure sodium-chloride solution. I concluded from this that we owe it to the calcium ions in the blood that our muscles do not twitch or beat rhythmically like our heart.[2]

To test this idea further, Mr. W. E. Garrey and I undertook, in 1899, a series of experiments, not yet published, on the behavior of muscles in solutions of sodium salts whose anions precipitate calcium. The muscle itself contains calcium salts, and we considered it likely that these calcium salts might help in preventing contractions. We therefore thought that by putting the muscle into solutions of sodium salts, which, by entering the muscle, precipitate the calcium contained in it, we might produce still more powerful rhythmical contractions than in a pure sodium-chloride solution. This was found to be true. In solutions of sodium-fluoride, -oxalate, -carbonate, -phosphate, etc., of the proper concentration (1 gram-molecule in 8 liters of the solution), we obtained similar, but more powerful, rhythmical contractions than in sodium-chloride solutions of the same osmotic pressure. Another series of observations confirms the idea that it is due to the calcium salts in our body that our muscles do not show any rhythmical contractions or twitchings. When we inject into the body of an animal any salts that are liable to precipitate calcium, we notice almost immediately twitchings of all the muscles.[3] It seems, therefore, rational that in the pathology of muscular twitchings the concentration of the calcium ions in the blood should be taken into consideration. It is quite possible that abnormal conditions may arise in the body which lead to an increase of such acids in the circulation as diminish the amount of calcium ions in the body, *e. g.*, oxalic acid, or others. The necessary outcome would be muscular twitchings. In that case the administration of calcium salts might cure the disease.

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[1] J. LOEB, *American Journal of Physiology*, Vol. III (1900), p. 327; Vol. V (1901), p. 362; Vol. VI (1902), p. 411. *Pflüger's Archiv*, Vol. LXXXVIII (1901), p. 68.

[2] *Festschrift für Fick*, 1899. See also S. RINGER, *Journal of Physiology*, Vol. VII (1886), p. 291. In this paper Ringer also mentions briefly the fact that Ba differs in its action from Ca and Sr.

2. In a recent paper I have shown that the antitoxic effects calcium produces when added to a pure NaCl solution are a function of its valency and the sign of its charge, inasmuch as similar effects can be produced by other bivalent or trivalent kations (*e. g.*, Mg, Sr, Ba, Zn, Fe, Co, Pb, Al, Cr), but not by bivalent or trivalent anions.[4] The question arises whether or not the inhibiting effects of Ca ions in the case of rhythmical contractions of muscles are also a function of the valency and electrical charge of the Ca ion. My earlier experiments were not opposed to such a conclusion. I had found that in $m/8$ solutions[5] of LiCl, NaCl, RbCl, and CsCl rhythmical contractions occur, while small amounts of the chlorides of Ca, Mg, Sr inhibit these contractions. I have since continued these experiments, with the following results: When we put a muscle (the gastrocnemius of the frog was used in these experiments) into a $m/8$ sodium-acetate solution, the twitchings of the muscle begin at once. The addition of from 3 to 4 c.c. of a m $CaCl_2$ solution to 100 c.c. of a $m/8$ sodium-acetate solution absolutely suppresses all twitchings. But even half the amount suffices for practical purposes, inasmuch as in this case only a few beats occur at the beginning. $MgCl_2$ and $SrCl_2$ act like $CaCl_2$. But $BaCl_2$ acts altogether differently. An addition of 5 c.c. of a m solution of $BaCl_2$ to 100 c.c. $m/8$ sodium-acetate solution not only does not stop the rhythmical contractions, but makes them more powerful. Instead of the rapid and rather weak fibrillary twitchings which occur in a $m/8$ sodium-acetate solution, more tetanic and energetic contractions occur when $BaCl_2$ is added. I then tried whether the muscle is able to beat in a pure $BaCl_2$ solution. It goes without saying that in pure solutions of $MgCl_2$, $CaCl_2$, and $SrCl_2$ a muscle does not show any rhythmical contraction. In a $m/8$ $BaCl_2$ solution, however, the muscle beat for forty minutes; in a $m/16$ solution, for one and a half hours; in a $m/32$ $BaCl_2$ solution, for over an hour; and in a $m/64$ $BaCl_2$ solution, for about half an hour. The beats showed the same tetanic form characteristic of the presence of barium. The fact that the beats stop sooner in a $m/8$ $BaCl_2$ solution than in a $m/16$ solution is due to the poisonous effects of barium. The fact that the beats stop also very soon in a

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[3] FRIEDENTHAL, *Engelmann's Archiv*, 1901, p. 145.

[4] *Pflüger's Archiv*, Vol. LXXXVIII (1901), p. 68; *American Journal of Physiology*, Vol. VI (1902), p. 411.

[5] By a $m/8$ solution is meant a solution which contains 1 gram-molecule in 8 liters.

$m/64$ $BaCl_2$ solution is due to the enormous absorption of water which occurs in such weak solutions.[6]

Similar facts were found for $Ba(NO_3)_2$ and $Ba(HO)_2$. The most striking fact is that the stimulating power of Ba salts is greater than that of the corresponding Na salts. In a $m/64$ $BaCl_2$ solution the muscle may begin to beat in a few minutes and may continue to do so for half an hour. I have even occasionally noticed rhythmical contractions in a $m/128$ $BaCl_2$ solution. But I have never noticed any rhythmical contractions of muscle in a $m/32$ NaCl solution, and as a rule even in a $m/16$ NaCl solution the twitchings begin only after a long latent period and last but a short time.

Barium is, however, not the only bivalent kation whose chloride possesses a higher stimulating power than that of the chloride of univalent kations. It was found that the chlorides of the heavy metals are also capable of producing rhythmical twitchings or beats of muscles in much lower concentrations than those found effective in the case of NaCl or LiCl. In $m/100$ solutions of $ZnCl_2$ strong beats occurred, which, however, did not last long on account of the rapid imbibition of the muscle with water, as well as on account of the poisonous effects of Zn. In stronger solutions than $m/64$ no beats occurred. The same was true for $ZnSO_4$. Solutions of $CdCl_2$ and $Pb(NO_3)_2$ also gave rise to a few contractions in the concentration of about $m/128$ to $m/150$.

The fact that the more concentrated solutions of the salts of heavy metals did not act is probably due to their poisonous effect. It is, therefore, evident that there are a number of chlorides with bivalent kations which are able to produce rhythmical contractions at a lower concentration than NaCl. It would, therefore, be wrong to ascribe the inhibiting effect of Ca salts upon rhythmical contractions to the double valency and the positive charge of the Ca-ions.

3. Does the effectiveness of salts for the production of rhythmical muscular contractions increase with the valency of the anion? This is decidedly not the case, as the following table shows. In this table are given the minimal concentrations of the solutions of various sodium salts in which rhythmical contractions occur:

	Salt	Minimal Effective Concentration
Univalent anions	NaCl	$m/16$
	NaBr	$m/16 - m/32$
	NaJ	$m/32$
	Na acetate	$m/32 - m/64$
	NaF	$m/64 - m/96$
	Na formiate	$m/80 - m/160$
Bivalent anions	Na_2 succinate	$m/16$
	Na_2SO_4	$m/32$
	$NaHCO_3$	$m/16 - m/32$
	Na_2 oxalate	$m/260 - m/300$
Trivalent anions	Na_2HPO_4	$m/128 - m/256$
	Na_3 citrate	$m/200$

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[6] The beats in $BaCl_2$ solution often do not begin at once, but after a latent period of from two to fifteen minutes.

It is obvious that the power of favoring rhythmical contractions in muscles is not an unequivocal function of the valency of the anion. It is likewise obvious that the sodium salts whose anions precipitate calcium powerfully, like sodium fluoride, sodium oxalate, and sodium phosphate, are among the most favorable salts to produce rhythmical twitchings. Sodium citrate does not precipitate calcium in the tissues, but prevents its precipitating other compounds, and therefore practically makes calcium inactive.[7] But that the precipitation and inactivation of calcium is perhaps not the only factor involved is shown by the efficiency of sodium formiate. It may be, however, that sodium formiate undergoes further changes in the tissues, and that one of the products formed acts upon calcium.

All these facts suggest that it might be worth while to test the idea whether or not the pathological cases of *muscular* hyperactivity and twitching are due to a lack of calcium in the muscles (or blood), and whether the evil can be mitigated by giving calcium salts to such patients. Experiments must be made in animals to find out whether or not such a treatment would do any harm before any therapeutical experiments in patients should be attempted. It is our intention to take up these experiments in the laboratory.

II. THE DIFFERENT EFFECTS OF CALCIUM IN THE CASE OF MYOGENIC AND NEUROGENIC RHYTHMICAL CONTRACTIONS

1. While it seems easy to suppress, by the addition of Ca, Sr, and Mg, rhythmical twitchings which originate in the muscle itself, the question arises whether the same means allow us to suppress, with equal ease, muscular twitchings which originate through the central nervous system. The simplest organism in which this can be tested is probably the jelly-fish. These animals contract rhythmically. Their central nervous system is contained in the margin of the swimming-bell, while the center of the animal is said to contain no nerve-elements except scattered neurons. By a cut parallel to the edge we can divide the animal into a marginal part, which contains the central nervous system, and a central part, without a central nervous system.

When this operation is performed, the margin will go on beating in sea-water, while the center will not beat. Romanes, who was (as I believe) the first to make this experiment, drew the conclusion that the central nervous system was the originator of the automatic contractions of this animal. From previous experiments of Aubert,[8] Howell,[9] and Greene[10] on the heart, and my own experiments on the muscles, I concluded that the center of a jelly-fish (Gonionemus) did not beat in sea-water on account of the presence of certain ions in sea-water, especially calcium, and I showed that the center of a medusa will beat rhythmically in a pure NaCl or NaBr solution.[11] The center of a medusa whose margin is cut off seems then to behave, to a certain extent, like the striped muscle. It was of some importance to find out how far this analogy goes. The following six solutions were prepared:

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[7] Sabbatani has shown that, although sodium citrate does not precipitate calcium, it renders it inactive. In the presence of a sufficient quantity of sodium citrate, calcium loses, *e. g.*, its coagulating effect; *Archives Italiennes de Biologie*, Vol. XXXVI (1901), p. 397.

[8] AUBERT, *Pflüger's Archiv*, Vol. XXIV (1881), p. 361.

[9] HOWELL, *American Journal of Physiology*, Vol. II (1898), p. 47.

[10] GREENE, *ibid.*, p. 82.

[11] LOEB, *ibid.*, Vol. III (1900), p. 383.

1. 100 c.c. $m/2$ NaCl
2. 100 c.c. $m/2$ NaCl + $\frac{1}{2}$ c.c. $\frac{5}{16}$ m $Ca(NO_3)_2$.
3. 100 c.c. $m/2$ NaCl + 1 c.c. $\frac{5}{16}$ m $Ca(NO_3)_2$
4. 100 c.c. $m/2$ NaCl + 2 c.c. $\frac{5}{16}$ m $Ca(NO_3)_2$
5. 100 c.c. $m/2$ NaCl + 4 c.c. $\frac{5}{16}$ m $Ca(NO_3)_2$
6. 100 c.c. $m/2$ NaCl + 8 c.c. $\frac{5}{16}$ m $Ca(NO_3)_2$

In solution 1 the center of a medusa begins at once to contract very rapidly. The velocity of contractions steadily increases, and very soon it becomes impossible to count the contractions. Occasionally the same happens in solution 2. But in the solutions 3 to 6 the center at first remains perfectly quiet. After a latent period of about ten minutes, often, but not always, contractions begin in solutions 3 to 5, or even in 6, which last sometimes as long as fifteen minutes. These contractions are not as rapid as those observed in a pure NaCl solution, and resemble more the normal contractions of a medusa in sea-water. A series of experiments was undertaken to find out the minimal amount of Ca required to prevent completely all contractions in a pure NaCl solution. In a mixture of 100 c.c. of a $m/2$ NaCl solution + 3 c.c. of a $\frac{5}{4}$ m $Ca(NO_3)_2$ solution no contractions occurred. A series of experiments with a slightly greater amount of $CaCl_2$ were made with the same result.

The same inhibitory effect can be produced if, instead of Ca, Sr or Mg is used. But Ba behaves altogether differently. The following solutions were tested:

100 c.c. $m/2$ $NaNO_3$
100 c.c. $m/2$ $NaNO_3$ + 1 c.c. m $BaCl_2$
100 c.c. $m/2$ $NaNO_3$ + 2 c.c. m $BaCl_2$
100 c.c. $m/2$ $NaNO_3$ + 4 c.c. m $BaCl_2$
100 c.c. $m/2$ $NaNO_3$ + 8 c.c. m $BaCl_2$
100 c.c. $m/2$ $NaNO_3$ + 16 c.c. m $BaCl_2$

When the center of a medusa was thrown into any of these solutions, the rhythmical contractions began at once. The center behaved as if the Ba ion had not been present, with this difference, however, that the solutions with a larger amount of barium were more poisonous than a pure NaCl solution. *Ba has, therefore, little or no inhibitory effect upon the center of a medusa.*[12]

The analogy between the effect of ions upon muscle and the center of a medusa goes still farther. I pointed out that possibly the Ca ions in the sea-water and the tissues of the medusa prevent the isolated center from beating in sea-water in the same way as the presence of Ca in the blood seems to prevent our muscles from beating. In order to test this idea, I added to the sea-water various salts which precipitate Ca, *e. g.*, NaF and Na_2HPO_4. I found that when a little more of these salts had been added than required to precipitate all the Ca in the sea-water, the center behaved indeed in the same way as if it had been put into a pure NaCl solution. When a little less Na_2HPO_4 was added, the beats began after a latent period, which varied according to the amount of Na_2HPO_4 added. Rapid contractions began at once when 32 c.c. of a $m/8$ Na_2HPO_4 solution was added to 68 c.c. of sea-water. The same result was obtained when 16 c.c. of a normal NaF solution was added to 100 c.c. of sea-water.

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[12] Since this was written I have received, through the kindness of Professor Sabbatani in Cagliari, a paper published by his assistant, Dr. Regoli, in which the latter shows that, while Ca and Sr diminish the irritability of the cerebral cortex, Ba has the opposite effect; REGOLI, "Azione dei metalli alcalino-terrosi sulla eccitabilità elettrica della corteccia cerebrale," *Bolletino d. Societate tra i Cultori delle Scienze etc. in Cagliari*, Torino, 1901.

The addition of about 13 c.c. of *m* sodium-citrate solution to 100 c.c. of sea-water also brought about immediate contraction of the isolated center. This salt does not bring about a precipitation of Ca in the sea-water or the tissues, but excludes the action of Ca ions in another way.

I did not succeed in bringing about such results with the addition of Na_2SO_4 to sea-water. Even the addition of 32 c.c. of *m* Na_2SO_4 to 100 c.c. of sea-water did not give rise to contractions, although the irritability of the center was increased. Experiments with the addition of $NaHCO_3$ remained also negative. But as only a few experiments were made with Na_2SO_4 and $NaCHO_3$, it is possible that a continuation of the work might lead to positive results.

It is, therefore, obvious that the centers can be caused to beat through a diminution of the amount of Ca they contain, and it may be further argued that the presence of Ca in the sea-water is the cause, or at least one of the causes, that prevent the centers from beating in sea-water.

It should, however, be added that, while a certain diminution of Ca in the center is necessary for the development of rhythmical contractions, the diminution has its limit. It appears that, if too much Ca is removed from the tissues, the beats will also cease. This is demonstrated by the following facts: When we put the center of a medusa into sea-water to which enough sodium citrate has been added, beats begin at once, last for a certain time, and then cease. If at this time the centers are put back into sea-water with less or no sodium citrate, beats will begin again. The explanation of this phenomenon seems to be as follows: The normal center of a medusa contains too much Ca for spontaneous rhythmical contractions. If we put a center into sea-water to which a large amount of NaF, Na_2HPO_4, or sodium citrate has been added, so much of the salt will diffuse at once into the organism that at least in the superficial cells enough Ca will be eliminated from the field of action to allow the spontaneous contractions to begin. Subsequently the same will happen in the deeper cells. The process of elimination of calcium in the cells proceeds, and very soon a period comes when the loss of Ca in all the cells will be too great for the contractions to go on. If, as soon as this occurs, the center is thrown into normal sea-water, or sea-water with only a little sodium citrate or phosphate, citrate and phosphate anions will diffuse back from the tissues into the sea-water, or Ca ions will diffuse into the cells, or both phenomena will occur, and beats will again begin. The same reasoning applies probably to the rhythmical contractions of muscles and the apex of the heart.

2. When we put the margin containing the central nervous system into a pure NaCl solution, it behaves very much like the center, *e. g.*, it begins to beat very rapidly, and the rapidity of the beats increases, at first steadily, until the poisonous effects of the pure NaCl solutions make themselves felt. But even the addition of large quantities of Ca does not inhibit these contractions. For instance, when we add from 2 to 5 c.c. of a $\frac{5}{4}m$ solution of $Ca(NO_3)_2$ to 100 c.c. of $m/2$ NaCl solution, the margin at once begins its rapid beats. The only effect the addition of calcium has is to make the rate of the beats a little slower than without calcium. I thought at first that the stimulus of the wound caused by the cutting off of the margin might be responsible for these contractions in the presence of calcium. But this is not the case, for if we put a whole Gonionemus intact into any of these solutions, it behaves like the isolated margin. The only possible inference is that the margin is much more immune toward the inhibiting effects of calcium than the center, a fact which I have pointed out already in a former paper.[13] In a pure $CaCl_2$ solution the margin will not beat.

Inasmuch as the essential difference between center and margin which accounts for this difference in the effect of calcium is the presence of the central nervous system in the margin, it may follow from these observations that for the suppression of twitchings of a nervous origin larger doses of calcium might be required than for the suppression of twitchings of muscular origin. Preliminary experiments on the motor nerves of frogs seem to harmonize with this idea. This suggests the possibility that, while a calcium treatment might be advisable for the cure of myogenic twitchings, for the suppression of neurogenic twitchings so much calcium might be required as to exclude the use of this remedy. This, too, is a point which further experiments on animals must decide before the matter may be tried in patients.

III. THE PRODUCTION OF HYPERSENSITIVENESS OF THE SKIN BY ELECTROLYTES

1. In a former paper I have shown that, aside from the rhythmical twitchings, the salts whose anions precipitate or inactivitate calcium also make muscles and motor nerves sensitive to stimuli which normally would not affect these organs. For example, when we put a fresh muscle for one or more minutes into a $m/8$ solution of sodium citrate, a peculiar form of irritability arises (contact irritability).[14] Whenever the muscle is taken out of the solution it goes into powerful tetanic contractions, which cease at once and give way to relaxation of the muscle as soon as the latter is put back into the solution. When this hypersensitive condition is once established, the contractions can be produced whenever the muscle is changed from any aqueous solution to any other non-aqueous medium, while the contractions cease when the muscle is put back into an aqueous medium, no matter whether the latter be a solution of an electrolyte or a non-conductor. It is rather striking that these phenomena do not occur when the above-mentioned solutions call forth at once the rhythmical contractions

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[13] *American Journal of Physiology*, Vol. III (1900), p. 383.

[14] *Ibid.*, Vol. V (1901), p. 362.

mentioned in the previous part of this paper. It almost looks as if there existed two allotropic states of the muscle substance, the one giving rise to rhythmical twitchings, the other to the peculiar tetanic contractions (contact reactions) just referred to.[15] Ultimately, however, in all cases rhythmical twitchings are produced.

As far as motor nerves are concerned, I have shown in the same paper that the same salts which produce this contact reaction produce hypersensitiveness of the nerve and ultimately rhythmical contractions of the muscle when acting upon the nerve alone.

It might be mentioned here in parenthesis that these facts may throw some light upon the action of cathartics. All the salts which give rise to the above-mentioned contact reaction or hypersensitiveness act as cathartics when introduced into the intestine. The common explanation of their action is the one which, I believe, was first suggested by Schmiedeberg, namely, that these salts prevent the absorption of liquids from the intestine, and that this retention of liquids causes the cathartic effect. I will not deny the effect of these salts upon the phenomena of absorption of water from the intestine,[16] but it is obvious from our experiments that the same salts must increase the irritability of the nerves and muscles of the intestine, and that this must facilitate the production of peristaltic motions, possibly through the mechanical or contact stimuli of the fæces upon the nerve-endings or the muscular wall of the intestine.

2. These experiments suggested the idea whether or not electrolytes are capable of producing also a hypersensitiveness of the skin and conditions that may be comparable to the conditions of hyperæsthesia or hyperalgesia. It is well known that when we suspend a pithed frog vertically so that its legs hang down, the latter will be lifted at once when they are dipped into an acid or alkali of a certain concentration, while no such reaction occurs when they are dipped into water. The reaction of the animal to acid may be so violent as to suggest to a layman the idea that it is suffering intense pain. I wondered whether by an alteration of the nature and proportion of ions in the skin the sensitiveness could be increased or varied in such a way as to make the skin as sensitive to the contact with pure water as it naturally is to strong acid. The experiments resulted in my finding certain solutions of electrolytes which did not seem to affect the animal directly, but yet made it extremely sensitive toward contact with water. The best solutions for this purpose are, as far as my present experiments go, $AlCl_3$ and sodium-citrate solutions. The way of proceeding is as follows: A number of solutions, say of $AlCl_3$, are prepared, namely, $m/32$, $m/16$, $m/8$, $m/4$, and possibly $m/2$. Then the weakest of the solutions is first brought in contact with the feet of the frog. If the feet are not withdrawn, the next stronger solution is used, and, if no reaction occurs, the next stronger. If one thus succeeds in keeping the feet of the animal for one minute or more in the $AlCl_3$ solu-

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[15] This difference is emphasized by the fact, found by my pupil, Dr. Zoethout, that an addition of potassium favors the contact reaction. As far as rhythmical contractions are concerned, K has an inhibiting effect.

[16] CUSHNY AND WALLACE, *American Journal of Physiology*, Vol. I (1899).

tion, subsequent contact of the feet with common tap-water or distilled water makes the animal act as if the water caused the most excruciating pain. The feet are violently withdrawn, rubbed against each other in a way that one notices otherwise only when the feet are dipped into strong acids. If the $AlCl_3$ solution chosen is too strong, the animal will not leave its feet in the solution, but will try to withdraw them. But in that case its attempts at withdrawing its feet from the solution are never as violent as the subsequent attempts at withdrawing the feet when brought into contact with common water. The stronger the solution of $AlCl_3$ is in which the feet had been kept, and the longer they had been in the solution, the stronger their sensitiveness toward water will become.

Sodium citrate acts very similarly to aluminium chloride. As the latter is slightly acid and sodium citrate slightly alkaline, the possibility was suggested that the H and HO ions are responsible for the hypersensitiveness. While it is possible to produce occasionally a slight hypersensitiveness toward common water by a pure solution of NaOH or HCl, the results are very unreliable. It is practically the same if one tries to use NaCl solutions to which slight and varying quantities of HCl or NaOH have been added. Better results can be obtained with solutions of oxalates, sulphates, carbonates, and phosphates. The sodium salts are preferable to the potassium salts, for the animal withdraws its feet much more rapidly from the solution of a potassium salt than from the solution of the corresponding sodium salt. This makes it difficult in the case of potassium salts to saturate the foot with the sufficient number of ions to induce the hypersensitiveness.

It goes without saying that the hypersensitiveness which can be produced by $AlCl_3$ and sodium citrate does not make itself felt toward water alone, but to salt solutions also. One can find a minimal concentration for each solution of an electrolyte at which a pithed frog almost instantly withdraws its feet when they come in contact with the solution. This minimal concentration is considerably lowered after a treatment of the foot with an $AlCl_3$ or sodium-citrate solution.

The production of hypersensitiveness is only one side of the problem. The mitigation of the hypersensitiveness is the other side. The violent reactions of a frog when its feet are dipped in tap-water after a treatment with $AlCl_3$ can be stopped instantly when the feet are put into a normal solution of cane sugar. When weaker solutions of cane sugar are used the feet are withdrawn, and the attempts at withdrawing become the more noticeable and violent the weaker the sugar solution is. Very concentrated solutions of urea, *e. g.*, 2n solutions, act similarly, but not so powerfully as cane sugar. Glycerine solutions gave no such results; neither have I been able to find as yet any solution of an electrolyte which acted this way. The fact that only very concentrated solutions of cane sugar or urea inhibited the hypersensitiveness gave rise to the idea that the diffusion of water out of the foot might be the inhibiting factor, and that a stream of diffusion in the opposite direction, namely, from the outside into the skin, might give rise to a withdrawal of the foot. The latter idea could be tested. When the feet of a pithed frog are dipped into a normal solution of cane sugar, they are not withdrawn, no matter how long they remain in the solution. But if subsequently (after several minutes) the feet are put into pure water, after a few (five to ten) seconds the feet are energetically withdrawn. In this case, obviously water diffuses into the skin, which previously had lost water.

There may be electrolytes which act similarly to cane sugar, but I have not yet found them. Every solution of an electrolyte causes, above a certain concentration, an immediate withdrawal of the feet, and this withdrawal is the more energetic the more concentrated the solution. This differs from the behavior of sugar and urea, which above a certain concentration have the opposite effect.

The lowest concentration at which the solutions of various electrolytes will cause a pithed frog to withdraw its feet instantly or in from five to ten seconds, is about as follows:

HCl,	$m/240$ or less	$CaCl_2$	$m/8$ or a little less
$NaOH$,	$m/80$ or less	$SrCl_2$	
$AgNO_3$,	$m/180$ or less	$BaCl_2$	
$FeCl_3$,	$m/60$ or less	$MgCl_2$	
$CdCl_2$	$m/32$ to $m/16$	KCl,	$m/8$ to $m/4$
$HgCl_2$		NH_4Cl,	$m/4$ to $3/8m$
$AlCl_3$,	$m/16$ to $m/8$	$NaCl$	$3m/8$ to $m/2$
		$LiCl$	

It almost looks as if the coagulating effect of the kations upon proteids was of some importance. The powerful effects of Ag, Cd, and Hg interfere somewhat with the conclusion that we are dealing with a pure valency effect, which otherwise seems to make itself felt. If, instead of the chlorides, the nitrates or sulphates of the same metals are chosen, the order of efficiency seems to remain practically the same, as far as can be judged from an as yet incomplete series of experiments.

As far as the anions are concerned, the order of efficiency is for the sodium salts about as follows:

Na_2 oxalate,	$m/8$	$NaHCO_3$	$3/8m$ to $m/2$
Na_3 citrate,	$m/8$	Na formiate	
Na_2SO_4,	$m/4$	Na_2 succinate	
$NaHPO_4$,	$m/4$	$NaCl$	
NaF,	$m/4$ to $m/2$		

In this case, as in the case of rhythmical contractions, the oxalates and citrates are the most powerful anions of this series. It is clear that, in the determination of the lowest concentration of a salt which is still able to cause the immediate withdrawal of the foot, one must remember that a number of solutions (*e. g.*, $AgNO_3$, $AlCl_3$, $FeCl_3$, HCl, $NaOH$, Na_3 citrate, etc.) have an after-effect which makes itself felt in an increase of irritability. Other solutions (*e. g.*, those of calcium salts) may possibly have the opposite effect, namely, to raise the threshold of stimulation for subsequent tests.

It was of some interest to ascertain whether the results in these experiments were produced through an action of the electrolytes upon the nerve-endings, or upon the nerves themselves. In experiments on frogs whose skin had been removed from the feet, the results described in this paper could not be produced. The experiment of putting the nerves themselves into the above-mentioned solutions remained practically without effect. It is possible that with solutions of much greater concentration results may be obtained. It is, therefore, certain that the results observed in our experiments are due to an action of the electrolytes upon the nerve-endings in the skin, and not to an action upon the sensory nerves.[17]

IV. CONCLUSIONS

The experiments mentioned in this paper were undertaken with two aims in view, a practical and a theoretical one. As far as the former is concerned, it follows from our investigations that abnormal muscular twitchings and contractions may be brought about in an organism by a reduction in the proportion of calcium (or magnesium) in the muscles or the blood, or an increase in the proportion of Na and other kations. In view of the fact that thus far no explanation has been found for pathological phenomena of this kind, it becomes of some importance to see whether or not in certain of these diseases the relative amount of calcium ions in the blood is diminished. If this should be the case, the administration of calcium would be the cure for these diseases, which thus far have been beyond medical control. It is also apparent from our experiments that for the suppression of neurogenic twitchings or contractions more calcium may possibly be required than for the suppression of myogenic twitchings. There has thus far been no clue as to the origin of hypersensitiveness or hyperalgesia of the skin. Our experiments show that slight variations in the proportion of certain ions in the skin can cause an enormous hypersensitiveness.

As far as the theoretical side of the paper is concerned, it was our aim to test the idea whether or not the "stimulating" and inhibiting effects of ions are an unequivocal function of their electrical charge or valency. Over a year ago I tested the same idea without being able to obtain positive results, and nothing was said about the subject in the paper in which the results were published.[18] The test was continued in the above-mentioned experiments, with results which, in my opinion, are equally questionable, if not altogether negative.

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[17] The chemical irritability of muscles is, as far as electrolytes are concerned, also greater than that of motor nerves. The reverse is true for electrical stimulation.

[18] *American Journal of Physiology*, Vol. VI (1901), p. 362.

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